invitrogen™

# Expanding Rat Glial Precursor Cells

You may expand Rat Glial Precursor Cells (GPCs) (Cat. no. N7746-100) as an adherent culture on CELLStart™ or poly-L-ornithine coated, tissue-culture treated flasks, plates or dishes. Harvest your cells when 70–80% confluent, before colonies start contacting each other. It is very important to strictly follow the guidelines for culturing Rat GPCs to keep them undifferentiated

## Materials Needed

- Culture vessels containing Rat Glial Precursor Cells at 70–80% confluency
- CELLStart™ (Cat. no. 10142-01) or poly-L-ornithine coated, tissue-culture treated flasks, plates, or Petri dishes
- Complete GPC growth medium*, pre-warmed to 37°C

  *Complete StemPro® NSC SFM (Cat. no. A1050901) supplemented with 2 mM GlutaMAX™-I (Cat. no. 35050-061) and 10 ng/mL PDGF-AA (Cat. no. PHG0035)
- Disposable, sterile 15-mL or 50-mL conical tubes
- 37°C incubator with humidified atmosphere of 5% $CO_2$
- Dulbecco's Phosphate Buffered Saline (D-PBS) without $Ca^{2+}$, $Mg^{2+}$, or phenol red (Cat. no. 14190-144)
- StemPro® Accutase® (Cat. no. A11105-01), pre-warmed to 37°C
- Hemacytometer, cell counter and Trypan Blue (Cat. no. 15250-061), LIVE/DEAD® Cell Vitality Assay Kit (Cat. no. L34951), or the Countess™ Automated Cell Counter (Cat. no. C10227)

## Protocol

**Note:** Rat GPCs readily stick to the plastic used in cell culture dishes and centrifuge tubes. Prior to use, rinse all material that will come in contact with the cells with medium to prevent cells from sticking to the plastic.

1. Remove the spent growth medium from the culture dish containing the cells, and store in a 15-mL tube to use as a washing solution.
2. Rinse the surface of the cell layer once with D-PBS without $Ca^{2+}$ and $Mg^{2+}$ (approximately 2 mL D-PBS per 10 $cm^2$ culture surface area) by adding the D-PBS to the side of the vessel opposite the attached cell layer, and rocking back and forth several times.
3. Aspirate the D-PBS and discard.
4. To detach the cells, add 3 mL of pre-warmed StemPro® Accutase® Cell Dissociation Reagent per T75 flask; adjust volume accordingly for culture dishes of other sizes.
5. Once you observe cell detachment, gently pipette up and down to break clumps into a single cell suspension. Stop the cell dissociation reaction by adding 7 mL of the spent medium from step 1. Disperse the medium by pipetting over the cell layer surface several times
6. Transfer the cells to a new 15-mL conical tube, pre-rinsed with medium, and centrifuge at 300 × *g* for 7 minutes at room temperature. Aspirate and discard the medium.
7. Resuspend the cell pellet in a minimal volume of pre-warmed complete growth medium and remove a sample for counting.
8. Determine the total number of cells and percent viability using your method of choice. If necessary, add complete growth medium to the cells to achieve the desired cell concentration and recount the cells.
9. Add complete growth medium to the tube containing cells so that the final viable cell concentration is $1 \times 10^4$ cells per µL.
10. Add the appropriate volume of cells to each CELLStart™ or poly-L-ornithine coated flask, plate, or Petri dish to the correct seeding density of $3 \times 10^4$ cells per $cm^2$.
11. Incubate cells at 37°C, 5% $CO_2$, and 90% humidity, and change growth medium every other day.

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